PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/10, C12Q 1/68, C12P 19/34, C07H 1/08 — A1

(11) International Publication Number: WO 97/41219

(43) International Publication Date: 6 November 1997 (06.11.97)

(21) International Application Number: PCT/GB97/01148

(22) International Filing Date: 25 April 1997 (25.04.97)

(30) Priority Data:
9608644.2 26 April 1996 (26.04.96) GB

(71) Applicant *(for all designated States except US)*: SCIENTIFIC GENERICS LIMITED [GB/GB]; Harston Mill, Harston, Cambridge CB2 5NH (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: POLLARD-KNIGHT, Denise, Vera [GB/GB]; 52 Harley House, Marylebone Road, London NW1 5HG (GB). MARTIN, Sophie, Elizabeth, Victoria [GB/GB]; 11 Catharine Street, Cambridge CB1 3AW (GB). WATSON, Susan [GB/GB]; 78 Stanley Road, Cambridge CB5 8LB (GB).

(74) Agent: SMART, Peter, J.; W.H. Beck, Greener & Co., 7 Stone Buildings, Lincoln's Inn, London WC2A 3SZ (GB).

(81) Designated States: CA, JP, KR, SG, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: ELECTRODE CAPTURE OF NUCLEIC ACID

(57) Abstract

Nucleic acid is captured from a mixture of cellular debris produced by cell lysis by exposing the mixture to an electrode and applying a nucleic acid capturing voltage to the electrode which is then removed from the mixture carrying said nucleic acid. The nucleic acid is undamaged and can be amplified by PCR.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

ELECTRODE CAPTURE OF NUCLEIC ACID

The present invention relates to processes for the manipulation of nucleic acids and in particular for capturing a nucleic acid or mixture of nucleic acids from cellular debris or other biomolecule mixtures.

When cells are lysed to release nucleic acids, the resulting mixture is complex. It may contain cell wall materials, proteins, polysaccharides and numerous other materials. To capture the nucleic acids contained therein has been a time consuming task which generally must be carried out before the nucleic acid can be used in other processes such as replication (or amplification) procedures or hybridisation assays.

Harrington et al in "DNA transformation with electrically charged tungsten microelectrodes", International Worm Meeting, abstract 240 disclosed that a tungsten microelectrode subjected to a voltage of 3 V would attract DNA from solution to enable the DNA to be introduced into nematode worms . The DNA remained on the electrode when the latter was withdrawn from the solution. Reversal of the voltage is mentioned as a method of displacing the DNA from the electrode, although this is not disclosed to be advantageous. The DNA was present in pure form in a suitable buffer.

We have now discovered that a similar method can be employed to remove DNA or other nucleic acids from the complex mixtures formed during cell lysis, i.e. from admixture with cellular debris or form mixtures with other biomolecules generally, and that the nucleic acids so obtained can be removed from the electrode without damage so that they may be used in subsequent processes. Accordingly, the present invention now provides a method for capturing nucleic acid from a mixture of said nucleic acid with other biomolecules, e.g. cellular debris, comprising exposing an electrode to said mixture and applying to said electrode a nucleic acid

attracting voltage, and removing said electrode from said mixture carrying said nucleic acid thereon.

The electrode may be removed from the mixture by physical movement of the electrode or by removal of the mixture, e.g. by washing.

A voltage of from 0 to 4, more preferably 0.5 to 3 volts is suitably applied to said electrode to attract said nucleic acid thereto. Generally, best results are obtained at approximately 1 V. Said electrode carrying said nucleic acid may then be exposed to a liquid into which said nucleic acid is to be introduced and said nucleic acid may be removed from said electrode into said liquid. This may be achieved by washing, preferably after reducing, turning off or reversing the electrical field. The voltage may be applied between a pair of electrodes which are both removed from the mixture, or only one electrode carrying said nucleic acid may be removed.

The removed nucleic acid may then be used as desired, e.g. subjected to a replication procedure or a hybridisation assay.

The mixture from which the nucleic acid is removed may be produced by a process of cell lysis as described in our PCT application PCT/GB95/02024. As described there, cells such as bacteria (e.g. E. Coli) may be lysed by subjecting them to a voltage of a few volts, e.g. 1 to 10 volts. Using the same electrode, the released nucleic acid may be captured and removed as described above. This provides a particularly elegant process for lysing cells and capturing nucleic acids from the cells.

Other crude mixtures from which to purify nucleic acids, especially DNA, include PCR or other amplification reaction mixes, sequencing reaction mixes, body fluid samples, e.g. blood or sputum or other DNA rich samples, e.g. micro-biological cultures.

For conducting processes such as nucleic acid amplification or hybridisation assays, it is generally necessary to denature DNA into single stranded form. As disclosed in WO92/04470, WO93/15224 and PCT/GB9500542, this

also may be achieved by applying a voltage to an electrode. Such methods of denaturation may be used in the further treatment of DNA captured by the methods described herein.

In the accompanying drawings;

Figure 1 is a gel produced in Example 1 as described below; and

Figure 2 is a gel produced in Example 2 as described below.

The present invention will be illustrated by the following examples.

Example 1

To illustrate the principle of the invention, DNA was captured from solution, transferred to a separate container and amplified by PCR to demonstrate the integrity of the DNA so captured.

A variety of voltages were applied to 1 $\mu g\ ml^{-1}$ of a 500 base pair lambda DNA in 1 x PCR buffer solution using a pair of blunt ended carbon electrodes. Captured DNA was removed from the solution on one of the electrodes and transferred to distilled water where reversal of the applied voltage was employed to displace the DNA into the water.

The transferred DNA was subjected to a conventional PCR procedure using the 500 bp fragment as template under the following PCR conditions:

60.5 μl transferred DNA in water
0.8 μM reverse primer
0.8 μM forward primer
2.5 U Taq polymerase
200 μM dNTP mix
buffer- 10 mM Tris.Cl (pH 8.3 at 25^{o}C), 50mM KCl, 0.1% gelatin
2.5 mM $MgCl_2$
water to 100μl

The resulting amplicons were run on a gel and the results are shown in Figure 1. The strongest amplification bands were achieved by adsorbing the template DNA onto the electrode at a voltage of +1 V. No amplification was seen when the DNA had been adsorbed at voltages of 4 or 8 V.

Example 2

The aim here was to capture transformed pBR322, released from lysed *E.coli* cells, on to an electrode surface and to confirm this by thermal amplification of a 410bp fragment of the plasmid.

E.coli cells containing pBR322 were grown up overnight at 37°C in LB broth containing 25 μg/ml ampicillin.

The cells (approximately 1 x 10^9 cfu/ml) were harvested by spinning down 100 ml of culture, resuspending in 40 mls, 10 mM Tris pH8.0 and further concentrating the cells into 10 mls 1 x PCR buffer. The stock culture was lysed by heating for 5 mins at 98°C and held on ice until use.

Voltages of between 0 and 4 volts were applied to 59.5 μl of the culture for 30 seconds using blunt ended carbon electrodes. Following the application of the field the electrodes were removed and placed into an equal volume HPLC grade water and the field reversed for 30 seconds to displace the DNA.

The water containing the displaced DNA was then used in a conventional thermal PCR reaction using the pBR322 plasmid as the template under the following conditions:

59.5 μl HPLC grade water containing the displaced DNA
0.4 μM forward primer
0.4 μM reverse primer
10 mM Tris. Cl(pH8.3 at 25°C), 50 mM KCl, 0.01%(W/v) gelatin
2.5 mM $MgCl_2$
200 μM of each dNTP

Two control experiments were carried out, one where the described procedure was carried out in the absence of the field to demonstrate the background following passive binding. The second was a thermal PCR carried out on the cell slurry, where 5 μl was used in a 50 μl reaction.

Thermal amplification was carried out for 20 cycles.

The resulting amplicons were run on a 1% ethidium bromide stained agarose gel.

Amplification is seen in Figure 2 following adsorption of the template on to the electrode at +1 and +2 volts. There is a smeared band present following adsorption of the DNA using +4 volts indicating degradation of the plasmid. Amplification is seen in the absence of the field but it is considerably lower than those where the voltage had been applied. No specific amplicon is observed where crude cell slurry was used as template, indicating that the presence of debris is inhibiting the reaction. This illustrates that the invention is also a method of purification as the plasmid (target) DNA has been preferentially removed from the slurry to give a clean and specific amplicon.

Many modifications and variations of the invention as described above may be made in accordance with the invention. For instance by using larger scale apparatus, particularly larger area electrodes, larger quantities of nucleic acid could be purified, enabling detection by less sensitive techniques than PCR. The techniques described herein may be used for concentrating nucleic acids by extracting them from a larger volume of liquid and releasing them into a smaller volume.

CLAIMS

1. A method for capturing nucleic acid from a mixture of said nucleic acid with other biomolecules, comprising exposing an electrode to said mixture and applying to said electrode a nucleic acid attracting voltage, and removing said electrode from said mixture carrying said nucleic acid thereon.

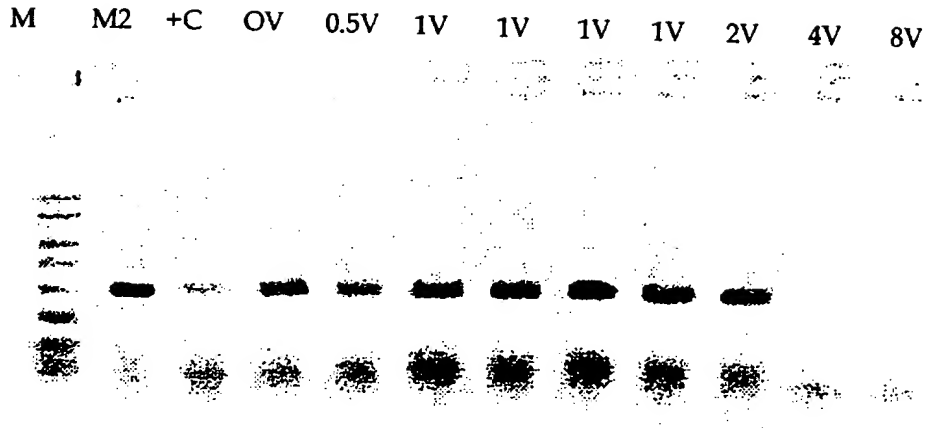
2. A method as claimed in Claim 1, wherein said nucleic acid is DNA.

3. A method as claimed in Claim 1 or Claim 2, wherein a voltage of from 0.5 to 3 volts is applied to said electrode to attract said nucleic acid thereto.

4. A method as claimed in Claim 1, wherein said electrode carrying said nucleic acid is exposed to a liquid into which said nucleic acid is to be introduced and said nucleic acid is removed from said electrode into said liquid.

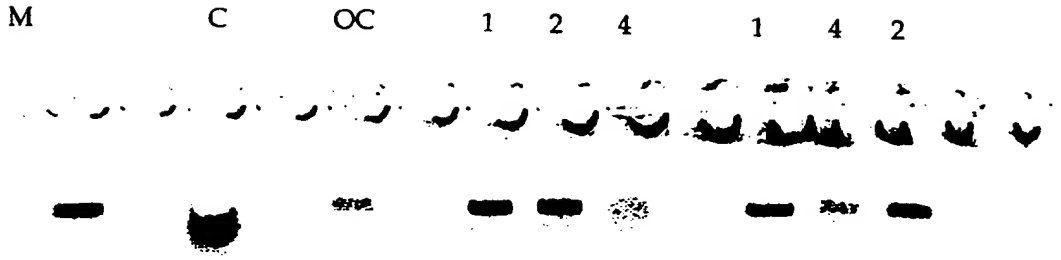
5. A method as claimed in any preceding claim, wherein the removed nucleic acid is subjected to a replication procedure or a hybridisation assay procedure.

6. A method as claimed in any preceding claim, wherein said other biomolecules comprise cellular debris.



M: marker; M2: 500bp marker; +C: PCR control; OV: with electrode, no current; 0.5-8V.

Fig 1



M	410bp marker thermally amplified from pBR322
C	PCR carried out on cell slurry using primers for 410bp from pBR322
OC	0 voltage applied
1	1 volt applied
2	2 volts applied
4	4 volts applied

Fig 2

INTERNATIONAL SEARCH REPORT

Interna' ·l Application No
PCT/GB 97/01148

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/10 C12Q1/68 C12P19/34 C07H1/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C12Q C12P C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 5 155 361 A (S.M. LINDSAY) 13 October 1992 see the whole document ---	1-4 5,6
X	BIOPHYSICAL JOURNAL, vol. 61, June 1992, NEW YORK US, pages 1570-1584, XP002038477 S.M. LINSAY ET AL.: "Potentiostatic deposition of DNA for scanning probe microscopy" see page 1570 - page 1573 see page 1582 - page 1584 ---	1-4
A Y	WO 92 04470 A (SCIENTIFIC GENERICS LTD) 19 March 1992 cited in the application see page 5, line 19 - page 6, line 17 --- -/--	1,2 5,6

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
22 August 1997	16. 09. 97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer De Kok, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interna ıl Application No
PCT/GB 97/01148

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANALYTICAL BIOCHEMISTRY, vol. 170 , no. 2, 1988, NEW YORK US, pages 421-431, XP002038478 E PALACEK: "Adsorptive transfer stripping voltammetry determination of nangram quantities of DNA immobilized at the electrode surface" see the whole document	1,2
A	WO 96 07917 A (NANOGEN) 14 March 1996 see figures 2,6 see page 13, line 31 - page 19, line 21 see page 8, line 31 - page 9, line 25	1-6
A	WO 95 34569 A (INVITEK GMBH) 21 December 1995 see abstract	1-6

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat l Application No
PCT/GB 97/01148

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5155361 A	13-10-92	US RE35317 E	27-08-96
WO 9204470 A	19-03-92	GB 2247889 A	18-03-92
		AU 8517391 A	30-03-92
		US 5527670 A	18-06-96
WO 9607917 A	14-03-96	US 5632957 A	27-05-97
		AU 3507095 A	27-03-96
		FI 970957 A	07-05-97
WO 9534569 A	21-12-95	DE 4422040 A	21-12-95
		DE 4422044 A	21-12-95
		DE 4447015 A	04-07-96
		EP 0765335 A	02-04-97